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MARINE POLLUTION BIOASSAY BY USING SEA URCHIN EGGS IN THE INLAND SEA OF JAPAN (THE SETO-NAIKAI)[1]

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With 4 Text-figures

Introduction

The Inland Sea of Japan (the Seto-Naikai), surrounded westerly by Kyushu Island, southerly by Shikoku Island, and northerly by Chugoku and Kinki districts of Honshu Island, has been very important in her long history of Japan for traffic, fisheries or recreation. Recently, however, the natural environments of this sea have been more and more damaged by pollution caused by wastes from rapidly increasing ships, industrial wastes and domestic sewages and also by practically uncontrolled reclamation of bays and inlets which might be very significant as the places for self-purification of the sea water or for feeding and spawning of fishes. Probably, it is now the last moment when all the efforts to conserve the nature of this sea for fisheries and recreation in future are effectual to some extent.

The first author proposed in 1971 the use of sea urchin eggs and embryos as indicator organisms in marine pollution bioassay by taking the normal fertilization, cleavage, permanent blastula or exogastrula as the indicatory stages or states, because the method is prominent in its simplicity, easiness, speed of treatment, high sensitivity, clearness of indicatory features, uniformity, and higher accuracy, and moreover different sea urchin species react to pollutants very similarly and this makes the same method available in any season of the year.

The present paper is to describe the pollution degree of respective regions, examined, of the Inland Sea of Japan according to the above-mentioned method as a part of the results of the Coordinate Investigations for Pollution in the Seto-Naikai. Here, we are going to present some standards, though arbitrary, as to the inhibitory

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effects of the polluted sea water in the fertilization and further development of sea urchin eggs, so that a kind of ranking in marine pollution becomes biologically possible.

Material and method

Anthocidaris crassispina (A. AGASSIZ) was the sea urchin experimented with. The fertilization, first cleavage, gastrulation and some anomalies in the development of eggs were checked in each test water. Eggs were obtained by the current KCl-method, washed several times with sea water, and were used as soon as possible, within 1 hour at the latest. Sperms were obtained from testes within 1 hour after being taken out of the test. The sperm density for insemination was standardized at about 1 dry sperm : 1,000 sea water in volume. When it was necessary, the preliminary check of eggs was done to see if the fertilization membrane was elevated in 3 minutes after insemination in over 85% of eggs and if the well synchronized first cleavage occurred in over 80% of them in the control laboratory water.

The collection of water samples in the Coordinate Investigations for Pollution in the Seto-Naikai was made in 16 days from July 20 to August 4, 1971 and about two hundred water samples thus collected were examined by bioassay. The water was drawn up with a plastic bucket from the surface layer or with a plastic reversing water bottle from the bottom layer at every station. The water collected at each station was, then, poured into a cleaned glass milk bottle and had been kept in the stock chest at $-20°C$ and later in the laboratory at $5°C$ till it was examined.

The bioassay of these water samples by using sea urchin eggs was made in 4 days from August 9 to 12 at the Seto Marine Biological Laboratory. In advance to the bioassay, the specific density of the sample water was measured by a hydrometor and was adjusted in need by adding Jamarin (commercial name for a raw material of artificial sea water) to ca. 1.024 (σ_{15}), the density general to the control running sea water of the laboratory. The eggs were inseminated in respective test water within 2–3 minutes as far as possible. Firstly the percent of eggs with the elevated fertilization membrane to the total eggs observed was read. The first cleavage occurred in most cases about 50 minutes after the insemination at $28°C$. Then, the rate and state of the first cleavage, namely proportions of undividing cells, normal two cells and multi-cells caused by polyspermy were checked at some adequate time. Two hundred eggs were fixed with 5% formaldehyde at a time for this examination. Lastly, the state of swimming embryos exclusive of those deposited on the bottom, namely proportions of permanent blastulae, normal gastrulae and abnormal exogastrulae was checked about 12 hours after the insemination. Two hundred embryos were fixed at a time for this check. The test was repeated 3 times on different batches as to the first cleavage, but was made only once as to further developmental stages to check the whole water samples in a limited time with a limited man power. In addition to the biological assay, the following three assays were carried out by other members of the party.

1. Chemical oxygen demand (COD) by general potassium permanganate method for saline water.
2. Amounts of heavy metals in the bottom mud, extracted with HCl and analized by atomic absorption spectroscopy.
3. Biomass and composition of benthos collected by an Ekman-Birge's bottom sampler scraping out 1/44 m^2.

Results of bioassay

During the field works of the investigations, ten and odd water samples were collected from the surface or bottom layers every day, thus about 200 samples were gathered in total. These samples were divided randomly to be tested at 15 times; and the running sea water of the laboratory was tested repeatedly as a control at every assay. Of the vast results thus obtained, those of 35 samples representing typically respective regions of the Inland Sea of Japan are described in detail in this paper, togather with the results of 2 control tests of the laboratory water.

The descriptions are made in 12 divisions separated from one another regionally.

1. Osaka Bay

Sample No. 20–2, from off Kobe Harbour. The effect of the bottom water upon eggs was great; the rate of fertilization was very low and the first cleavage did not occur completely. Big amounts of Zn and Pb were contained in the bottom mud and the benthos was represented by only a few polychaetes. This station seems to be heavily polluted.

2. The Sea of Harima

Sample No. 20–6, from the estuary of the River Ichi. The surface water affected the fertilization considerably and the first cleavage somewhat differently; unfortunately further development was not checked. The bottom water inhibited the both processes much more heavily. The water showed a high value of COD and the bottom mud contained a high level of Zn, Cr and Pb. The benthos was represented by scarce polychaetes.

Sample No. 21–4, from the Cove of Iwami. The water from either of the surface and bottom layers did not show any significant inhibition to the fertilization, first cleavage and gastrulation, though these were slightly suppressed by bottom water. The value of COD was a little higher in the lower layer. The benthos consisted of abundant polychaetes. The results may indicate a clean state of the water at this station.

Sample No. 21–9, from the entrance to Kojima Bay. The surface water affected considerably earlier stages of the development, though the later the less effective. On the other hand, the bottom water was a little less effective. The COD value was much higher in the surface than in the bottom layer; the mud contained a big amount of Zn, the benthos, however, consisted of abundant crustaceans.

Sample No. 3–1, from the southern part of the sea. The bottom water allowed the

normal fertilization, cleavage and gastrulation. The COD value was very low, but the benthic biomass was not so big.

3. The Sound of Mizushima

Sample No. 22–4 from the entrance to the Sound of Mizushima, rough water. The surface water did not inhibit significantly the fertilization, first cleavage and gastrulation. The bottom water did not arrest significantly the fertilization, either, but considerably affected the first cleavage, though the later development went on almost normally.

Sample No. 22–14, from off Mizushima. The bottom water inhibited more or less the fertilization and more strongly the first cleavage; the gastrulation occurred rather regularly, but the development was slightly delayed.

Sample No. 22–22, from off Konko. The bottom water affected more or less the fertilization and significantly the first cleavage. The rate of regular gastrulation dropped to about 50%, one half remained in permanent blastulae or showed some delay of development. The bottom mud contained some amount of Zn and the benthos consisted mainly of many crustaceans.

Sample No. 23–2, from the estuary of the River Takahashi. The rate of fertilization was lowered to 50% and the first cleavage was inhibited nearly completely in the bottom water. The benthos on the muddy floor was scarce.

4. The Sea of Bingo.

Sample No. 23–4, from off Fukuyama. The water from either of the surface and bottom layers suppressed the fertilization completely, though the COD value was rather low in both layers. The benthic biomass was small.

Sample No. 2–3, from Sakaide Harbour. The bottom water seemed to allow the normal fertilization, cleavage and gastrulation. The COD value was low. The benthos consisted mainly of many polychaetes.

5. The Sea of Hiuchi.

Sample No. 31–6, from off Niugawa. The bottom water allowed the fertilization nearly wholly, but the rate of regular first cleavage was somewhat lowered in it by significant appearance of polyspermic cleavage, and further the gastrulation was almost inhibited or delayed, a significant number of exogastrulae being observed. The COD value was low, but no benthic animals were observed.

Sample No. 1–3, from off Iyo-Mishima. The surface water inhibited the fertilization considerably and the first cleavage nearly completely. On the other hand, the bottom water allowed the regular fertilization, cleavage and gastrulation. The COD value was much higher in the surface than in the bottom layer. The mud contained a considerable amount of Zn and no benthos on the floor.

Sample No. 1–6, from off Toyohama. The bottom water more or less inhibited the fertilization and much more strongly the first cleavage, with a significant occurrence of polyspermic cleavage; the gastrulation went on rather well but was more or less delayed. The COD value was low. The benthos consisted mainly of polychaetes.

Sample No. 1–12, from off Kawanoe. The bottom water hardly inhibited the fertilization, but very significantly the first cleavage, so that about a half of eggs remained in the one-cell state. However, the gastrulation went on rather regularly. The COD value was low. The bottom mud contained a big amount of Zn and the benthos consisted mainly of many bivalves.

6. The waters around the Geiyo Islands.

Sample No. 24–2, from off Mihara. In the bottom water the rate of successful fertilization dropped to 50%, the first cleavage was more affected, and the gastrulation was inhibited much more strongly, but the COD value was not so high. The bottom mud contained large amounts of Zn and Pb, and the benthos consisted of a small number of polychaetes.

Sample No. 24–5, from off Chigirijima Isle. The surface water inhibited very significantly the fertilization and first cleavage. The gastrulation was affected as heavily in it, as about one quarter of embryos showed an exogastrulation and some delay of development. The bottom water, however, hardly arrested these processes. The COD value was higher in the surface than in the bottom layer, though the values were rather low. The benthos was scarce.

7. The Sound of Aki.

Sample No. 25–2, from off Kure. The bottom water more or less inhibited the fertilization and first cleavage, and almost arrested the gastrulation. The bottom mud contained great amounts of Zn and Pb, however, the benthic biomass was very large, consisting mainly of polychaetes.

Sample No. 25–8, from off Otake. The water from either of surface and bottom layers arrested more or less the fertilization, and the bottom water inhibited the first cleavage more strongly than the surface water, but further development went on rather regularly. The COD value was very high in the surface water. The bottom mud contained a surprisingly great amount of Zn and no aminals were found in it.

8. The Sea of Iyo.

Sample No. 30–5, from off Matsuyama. In the bottom water, the fertilization was done successfully in over a half of eggs, but the first cleavage occurred in less than a half of them. The gastrulation was inhibited a little less than earlier stages. The COD value was low. The bottom mud contained large amounts of Zn and Cu, and some benthic animals mainly consisting of polychaetes.

9. The Sea of Suo.

Sample No. 26–9, from Mitaziri Bay. The surface water inhibited both the fertilization and first cleavage, almost wholly the former and completely the latter. In the bottom water the fertilization proceeded nearly normally, but the rate of the first cleavage decreased significantly, though the inhibition of gastrulation became much less. The COD value was very high in both water layers, especially it was enormously higher in the surface water. The bottom mud contained a very large amount of Zn and con-

siderable amounts of Pb and Cu. The benthos was quite absent.
Sample No. 27–1, from off Ube. The surface water suppressed the fertilization by a little less than 50% of eggs and arrested the first cleavage by more than 50%; some occurrences of polyspermic cleavage were noted. The gastrulation went on almost regularly, but delayed significantly. On the other hand, the bottom water allowed the normal fertilization, but it decreased the rate of the first cleavage and gastrulation, especially remarkably the latter. The COD value was low, but a little higher in the surface than in the bottom layer. The bottom mud contained a big amount of Zn, but no living animals.

10. Beppu Bay.

Sample No. 29–5, from off Tsurusaki. The bottom water scarcely arrested the fertilization, but somewhat inhibited the first cleavage, inducing significantly the polyspermic cleavage. The deformed gastrulation was noted. The COD value was low. The bottom mud contained a large amount of Zn and the benthos consisted of many polychaetes.

11. The Bungo Channel.

Sample No. 29–6, from off Saganoseki. The bottom water allowed the regular fertilization and first cleavage, but somewhat arrested the gastrulation. The COD value was low. The bottom mud contained very large amounts of Zn, Pb, Cr, Ni and also a significant amount of Cu, but a small number of benthic crustaceans were found on the floor.

12. The Kii Channel.

Sample No. 3–4, from off Komatsujima, rough water. The fertilization, first cleavage and gastrulation proceeded almost normally in the bottom water. The COD value was very low, though the bottom mud contained large amounts of Zn and Ni; the benthos was scarce.

Control.

Sample Nos. C–9, 10, the runninng sea water of the laboratory. In the surface water near the Seto Marine Biological Laboratory the fertilization, first cleavage and gastrulation proceeded quite normally.

Considerations

During the Coordinate Investigations for Pollution in the Seto-Naikai, the sample water was treated chemically only to see the COD, chemical oxygen demand. Generally the COD value was higher in the surface than in the bottom layer. The water with very high COD values actually inhibited strongly the fertilization, first cleavage and gastrulation. In the water with lesser COD values, however, the inhibitory degree was never correlated with the COD value, as seen throughout the results. Some water samples with lower COD values recorded very strong inhibitory effects on the fertilization, first cleavage and gastrulation, while some others with relatively

higher COD values allowed rather regular fertilization and further development. Therefore, except for water samples with much higher COD values, it is hardly possible to guess the degree of inhibitory effects on the fertilization and further development by COD value of the water tested. Much more data will be needed to see how the COD value is reflected on the biological phenomena mentioned above.

As seen clearly in Table 1, respective water samples affected differently at different developmental stages; some allowed the fertilization nearly wholly, but arrested very significantly the first cleavage, while some others inhibited strongly the fertilization, but the first cleavage and further development of fertilized eggs went on in a much less affected state. This must be attributed to differences in the nature and amount of pollutants. It is very regrettable that no other data else than COD were available as to the chemical nature of the water. So far as the very limited data are concerned, the amount of heavy metals deposited in the bottom mud seems to be correlated with the induction of permanent blastula or exogastrula as it was observed for instances in areas around metal refinary works at Chigirijima and Saganoseki. However, we are not sure whether or not the elements in the water, which brought about such biological effects, were the same as those heavy metals, listed here, deposited in the bottom mud.

The benthic faunas at surveyed stations consisted in most cases mainly of polychaetes. And the size of benthic biomass seemed to be more or less correlated with the degree of inhibitory effects at least in the bottom water. In this case, however, it must be kept in our mind that the nature and amount of benthos will differ much with the nature of sediments, inclusive of grain size and water flow near the bottom.

It is needless to mention that there are some gaps between the surface water and the bottom water very near the floor, in the degree, time of the strongest effect, and the mode of effects. But, if it is generally accepted in such shallow places as the observed stations in the Inland Sea that the pollution in the surface water will be reflected in the benthos, the benthic fauna must be regarded as the result of synthetic and accumulated effects of pollutants in a long term at least equivalent to the life span of constituent animals. In a way, the benthos may be accepted as an indicator of the chronic injuries by pollutants. In contrast with this, the bioassay by the earlier developmental stages of sea urchin eggs will be significant only as a judgement for the acute injuries by pollutants. Then, it is possible that the effects of any pollutants which will work very weakly and slowly, but so steadily as to bring animals to death ultimately, might not be noticed in the present bioassay. The effects of any pollutants which will affect the physiological processes appearing only in animal structures of very high organization, might not be noticed in this way, either.

When Okubo and Okubo (1962) proposed the use of developing eggs and embryos of sea urchins and bivalves as indicator organisms for the sea water pollution, they cleared that the maximal concentrations of *some* pollutants ineffective to induce any morphological anomalies in them were seemingly directly equal to the safety level

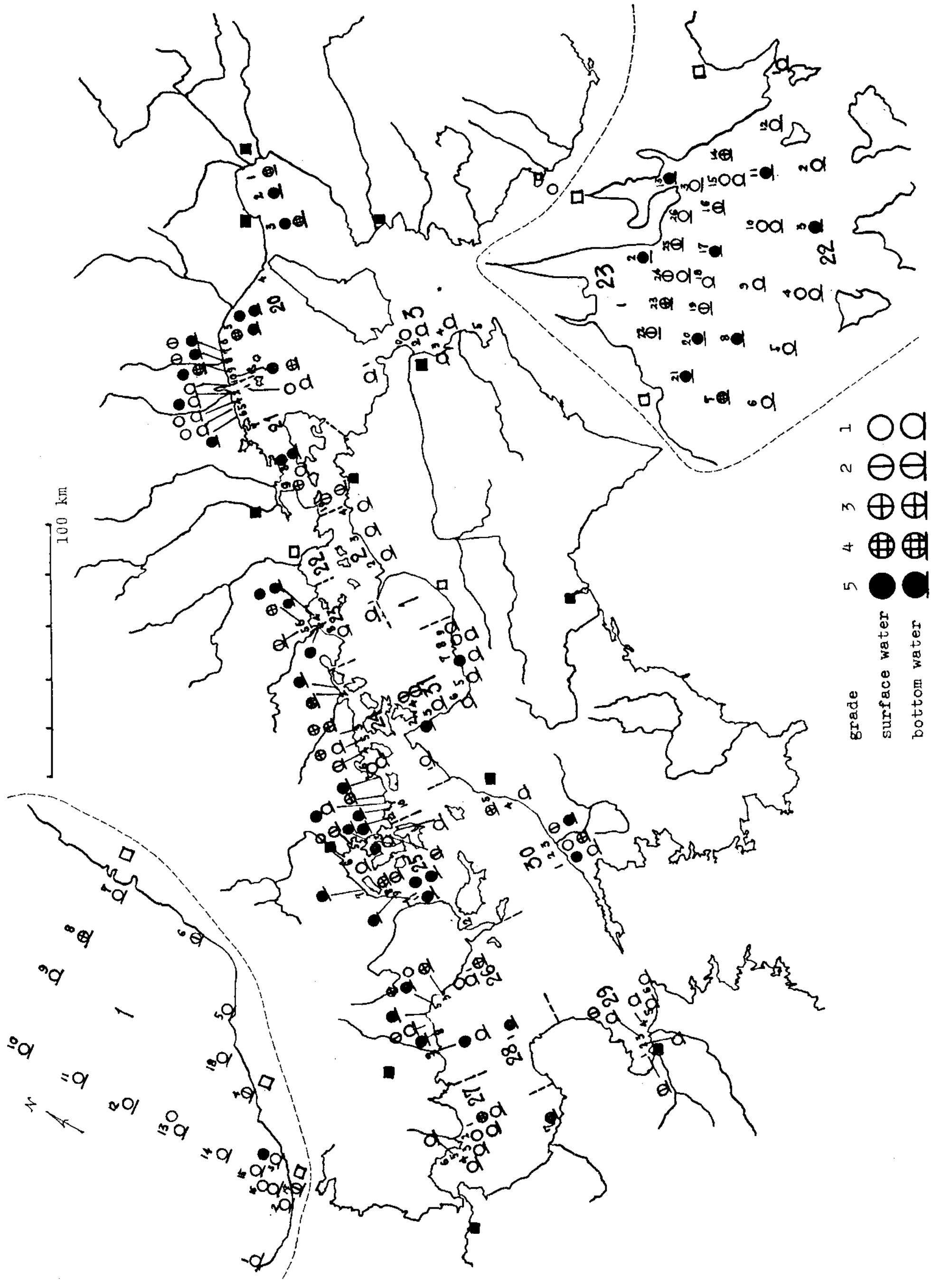

Fig. 1. Pollution map of the Inland Sea of Japan, showing stations by numbers and the inhibitory degree of the site water upon the fertilization and further development of sea urchin eggs at respective stations by signs. Inhibitory grades on the fertilization of the sea urchin eggs. Larger mumerals indicate the date of water collection.

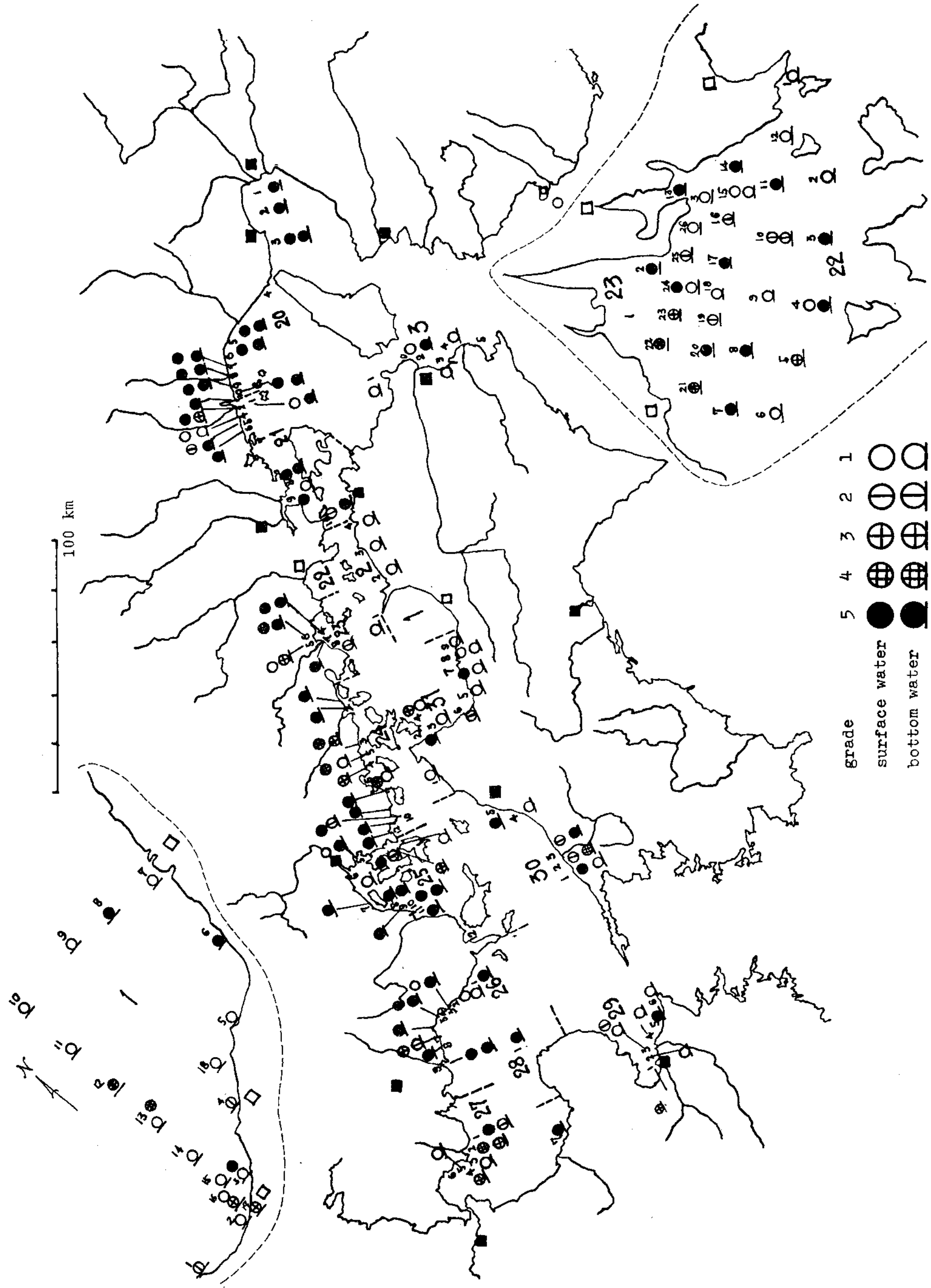

Fig. 2. Pollution map of the Inland Sea of Japan, showing stations by numbers and the inhibitory degree of the site water upon the fertilization and further development of sea urchin eggs at respective stations by signs. Inhibitory grades on the first cleavage. Larger numerals indicate the date of water collection.

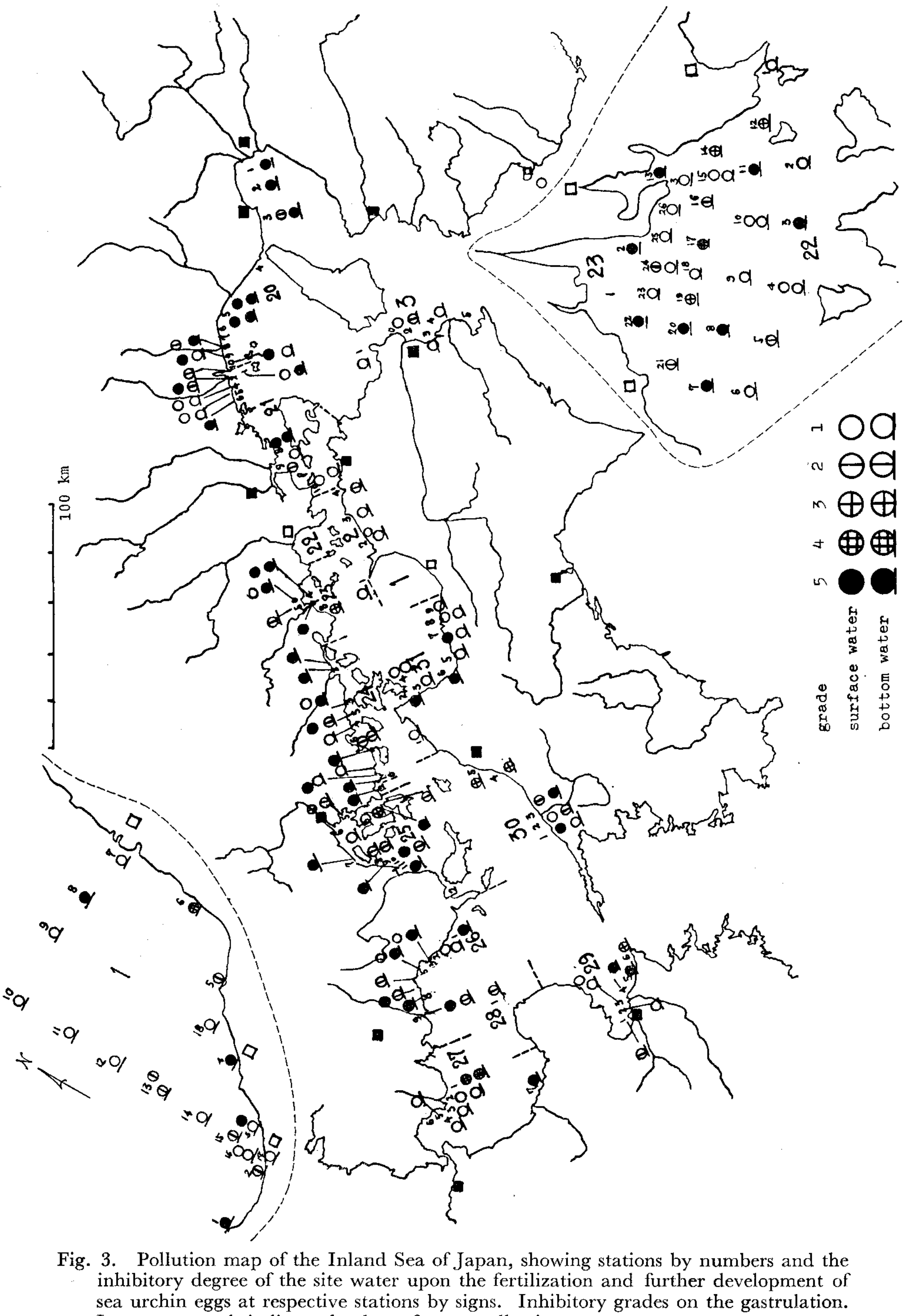

Fig. 3. Pollution map of the Inland Sea of Japan, showing stations by numbers and the inhibitory degree of the site water upon the fertilization and further development of sea urchin eggs at respective stations by signs. Inhibitory grades on the gastrulation. Larger numerals indicate the date of water collection.

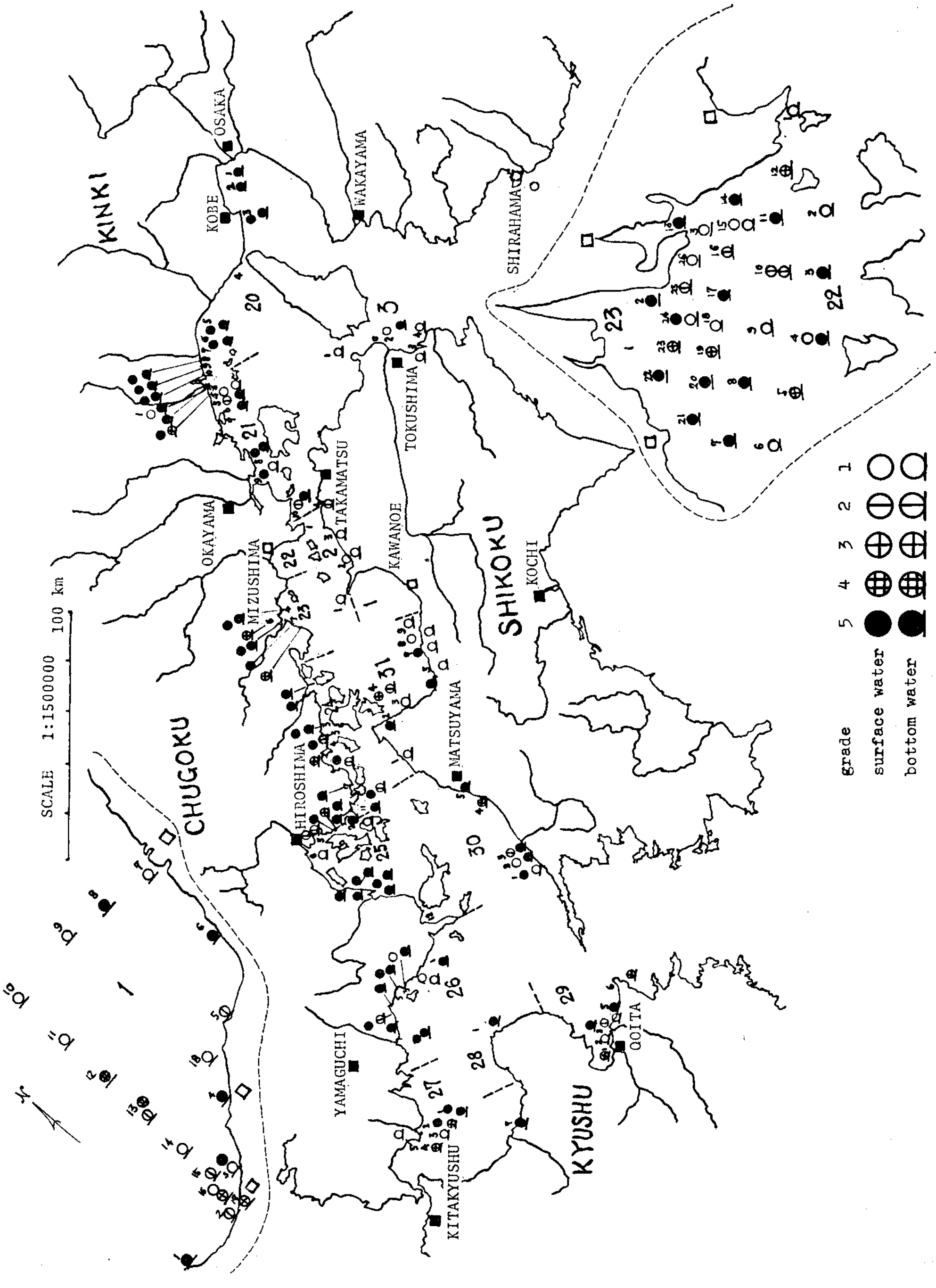

Fig. 4. Pollution map of the Inland Sea of Japan, showing stations by numbers and the inhibitory degree of the site water upon the fertilization and further development of sea urchin eggs at respective stations by signs. Inhibitory grades throughout the three early developmental stages. Larger numerals indicate the date of water collection.

(48 hrs. TLm×0.1) of the pollutant concentration for littoral fishes. WOELKE (1967), who recommended the toxicity measurement of water quality with the bioassay using embryos of Pacific oyster, warns us to be very cautious to extend the results obtained using some animal to other orgaisms, but at the same time he is expecting that some parallelism will be seen between the results obtained by using very different organisms.

We never think that the results of the bioassay using the earlier developmental stages of sea urchin eggs are applicable to every other organism and to every kind of pollutant. However, the results obtained by this method (KOBAYASHI 1971) agree almost to those given by OKUBO and OKUBO. Then, the present simple method may be significant to some extent to show in a limited time the general tendency of biological effects of sea water pollution in such a wide area as the Inland Sea.

Theoretically, there must be some questions to compare the polluted water samples of different natures with one another all at the same level. But, at the same time, it is quite evident that some general comparison of the biological effects between these samples can be allowed in some ways. At present, it must be the most important problem how the different effects are classified and then ranked. And a quite arbitrary ranking is proposed here tentatively as seen in Table 2. Respective water samples were checked by the rate and degree of the fertilization, first cleavage, gastrulation and such abnormalities as polyspermic cleavage, permanent blastula, exogastrula, stop of development in earlier stages, delay of development, and any kind of deformations, and were graded into the violently (5), strongly (4), moderately (3) and weakly (2) inhibitory sea water and the non-inhibitory ordinary (1) sea water. The least rate of respective normal developmental processes to define the (5) violently inhibitory water is set at 50% as ID50 (inhibitory degree 50%) somewhat comparable to LD50 (lethal dose 50%). Ranking of grades 4 to 1 is made quite arbitrary at every 10 to 15% of the rate of respective developmental processes and states, excepting the cases of polyspermic cleavage and exogastrula, which occurred rather infrequently and then it needs a quite special standard to make ranking of the frequency of occurrence of these anomalies. The inhibitory degree at the fertilization, first cleavage and gastrulation is generally judged respectively on the lowest (for normal processes) or the highest (for abnormal states) observed rate of their occurrences, of course some exceptionally low or high rates are excluded. And lastly, the inhibitory degree throughout the whole early developmental stages is settled by the heaviest effect in any of these stages, as this will decide the rate of survival. Text-figures 1–4 show the results of ranking tentatively done in the above mentioned way, fig. 1 for the inhibitory degree at the fertilization, fig. 2 at the first cleavage, fig. 3 at the gastrulation, and fig. 4 for the general inhibitory degree throughout the three stages checked. At these figures, it will be seen at a moment how the sea water in Osaka Bay, for instances, is polluted heavily as compared with the running sea water of the Seto Marine Biological Laboratory.

Throughout the whole examinations, it seems that the natural unpolluted state of the *water* is better conserved in the bottom layer. Therefore, in future, it is urged to make clear the relationship between the inhibitory effects and the pollutants in the

surface water at respective stations by close chemical analyses of the water and also by improvements of the method of bioassay itself.

We wish to express our hearty thanks to the staff of the Seto Marine Biological Laboratory for a fund and the facilities given to us in carrying out the reseaches at the laboratory, particularly to Dr. Takashi TOKIOKA for his kind advices and criticism. Our deep gratitude is due to the members of the Coordinate Investigations for Pollution in the Seto-Naikai, who helped us obtain water samples and permitted us to refer to some of the results of their researches in advance to publication.

Summary

1. A series of bioassays were made to examine the inhibitory degree of the polluted sea water in the Inland Sea of Japan upon the fertilization and further development of sea urchin eggs, as a part of Coordinate Investigations for Pollution in the Seto-Naikai, July 20 to August 4, 1971. The water samples were tested at the Seto Marine Biological Laboratory in 4 days from August 9 to 12.

2. The indicatory stages and states were the fertilization, first cleavage, gastrulation and such anomalies as polyspermic cleavage, permanent blastula and exogastrula.

3. The inhibitory effects on the fertilization, first cleavage and gastrulation were not regularly correlated with the COD values; a positive relation was seen only at very higher values of COD.

4. The relation between heavy metals in the bottom mud and inhibitory effects of the bottom water might be possible, as the induction of permanent blastula or exogastrula was noted in areas around metal refinary works at Chigirijima and Saganoseki.

5. The benthic fauna in surveyed areas in the Inland Sea of Japan consists in most cases mainly of polychaetes. Some relation between the benthos and inhibitory effects can be thought of.

6. The results of bioassay by sea urchin eggs may be taken as an expression of some direct effects (acute injuries) of the sea water tested, while the benthos as a reflection of the sea condition in a long term (chronic injuries).

7. The ranking of inhibitory degrees of polluted water samples upon the fertilization and further development of sea urchin eggs is proposed tentatively as seen in Table 2.

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Table 1. Results of the bioassay of the sea water pollution in the Inland Sea of Japan using *Anthocidaris crassispina* eggs. Date: August 9–12, 1971. Test water temperature 28°C. Of the sample number, the first half shows the date of sample collection, 20 to 31 are of July and 1 to 3 are of August, C shows the control. Numerals in circle show the degree of inhibitory effects of polluted water on the development of sea urchin eggs. In the column of benthos, **Crus.** shows that the benthos consists mainly of crustaceans, and similarly **Mol.** and **Poly.** indicate respectively molluscs and polychaetes. *Cr: values somewhat doubtful.

Sample No.	Location (depth)	Fertiliz.	First cleavage			Gastrulation			Other notes				
		membrane formation	1 cell	2 cell (normal)	multi-cell (polyspermy)	permanent blastula	gastrula (normal)	exo-gastrula	abnormal develop.	COD	heavy metals	benthos	specific density
	(m)	%	%	%	%	%	%	%		ppm	ppm		
20–2	Off Kobe Harbour	8	100	0	0						Zn 323 Pb 103 Cu 60		
⑤	Bottom	⑤ 0.5	100	⑤ 0	0						Cr 58 Cd 1.8	10 Poly.	1.0156
	(16.7)	2	100	0	0						Ni 25 Hg 0.08		
20–6	Estuary of	20	82	18	0	—	—	—					
⑤	River Ichi	④ 65	68	⑤ 31.5	0.5								1.0150
	Surface	58	65	34.5	0.5								
		5	95.5	4.5	0						Zn 475 Pb 123 Cu 88		
⑤	Bottom	⑤ 7.5	99	⑤ 1	0					3.8	Cr 550 Cd 2.3	14 Poly.	1.0131
	(11.0)	8.5	99	1	0						Ni 20 Hg 0.07		
21–4	Cove of	100	7.5	92.5	0	2.5	97.5	0					
①	Iwami	① 100	3	① 97	0		①			2.4			1.0091
	Surface	100	4	96	0								
		97	5	94.5	0.5	1	99	0				101	
①	Bottom	① 96	6.5	① 93	0.5		①			2.6			1.0194
	(6.0)	96	7.5	91.5	1							Poly.	

21–9 ⑤	Entrance to Kojima Bay Surface	77 ③ 64.5	79.5 88.5	20.5 ⑤ 11.5	0 0	6.5	93.5 ②	0		6.9			1.0029
①	Bottom (1.5)	91 ① 94.5 93	14 8 9.5	86 ① 92 90.5	0 0 0	0.5	99.5 ①	0		3.7	Zn 210 Pb 55 Cu 46 Cr 28 Cd 2.0 Ni 23 Hg 0.17	191 Crus.	1.0108
3–1 ①	Southern part of Sea of Harima Bottom (28.0)	99.5 ① 99.5	6 3.5	94 ① 96.5	0 0	2	98 ①	0		1.1	Zn 75 Cu 18 Cr 38 Cd 1.3 Ni 39	4 Poly.	1.0205
22–4 ①	Entrance to Sound of Mizushima Surface rough water	89.5 ① 93.5 97.5	13.5 11 4	86.5 ① 89 96	0 0 0	1	99 ①	0					1.0194
⑤	Bottom (12.0)	93 ① 91 93.5	88 83 80.5	12 ⑤ 16 19	0 1 0.5	4	96 ①	0			Zn 49 Cu 6 *Cr 8 Cd 1.8 Ni 21 Hg 0.03		1.0210
22–14 ⑤	Off Mizushima Bottom (20.0)	84 ③ 64.5 69.5	65.5 65 56	34 ⑤ 35 42.5	0.5 0 1.5	5.5	94.5 ②	0	slightly delay ③		Zn 73 Pb 25 Cu 11 *Cr 10 Cd 2.0 Ni 14		1.0172

Table 1. (continued)

Sample No.	Location (depth)	Fertiliz. membrane formation	First cleavage 1 cell	2 cell (normal)	multi-cell (polyspermy)	Gastrulation permanent blastula	gastrula (normal)	exo-gastrula	Other notes abnormal develop.	COD	heavy metals	benthos	specific density
	(m)	%	%	%	%	%	%	%		ppm	ppm		
22–22 ⑤	Off Konkô Bottom (3.8)	76.5 ② 76 71	44 45.5 40.5	56 ④ 54.5 59.5	0 0 0	51	49 ⑤	0	delay ④		Zn 165 Pb 50 Cu 35 Cr 38 Cd 2.5 Ni 35 Hg 0.02	75 Crus.	1.0199
23–2 ⑤	Estuary of River Takahashi Bottom (2.5)	51 ⑤ 47.5 55.5	100 100 95.5	0 ⑤ 0 4.5	0 0 0							18	1.0168
23–4 ⑤	Off Fukuyama Surface	0 ⑤ 0 0	100 100 100	0 ⑤ 0 0	0 0 0					2.3			1.0193
⑤	Bottom (15.0)	0 ⑤ 0 0	100 100 100	0 ⑤ 0 0	0 0 0					1.8		10 Mol.	1.0193
2–3 ①	Sakaide Harbour Bottom (13.0)	92.5 ① 96 96.5	13.5 7 8	85.5 ① 92.5 91.5	1 0.5 0.5	0.5	99.5 ①	0		1.5		43 Poly.	1.0193

31–6 ⑤	Off Niugawa Bottom (7.2)	96.5 ① 85.2 93	8.5 24 9	82 ② 70.5 84	9.5 ③ 5.5 7	70.5	13.5 ⑤	16 ⑤	delay ④	3.0	Pb 73 Cu 75 Cr 25 Cd 3.0 Ni 28 Hg 0.06	0	1.0210
1–3 ⑤	Off Iyo- Mishima Surface	22.5 ⑤ 26 31	99.5 97 96	0.5 ⑤ 3 4	0 0 0					104			1.0206
①	Bottom (16.5)	97 ① 93.5 98	5.5 9 3.5	94 ① 89.5 95.5	0.5 1.5 1	1	99 ①	0		3.9	Zn 318 Pb 68 Cu 80 Cr 33 Cd 3.3 Ni 37	0	1.0222
1–6 ⑤	Off Toyohama Bottom (8.0)	73 ② 77 94.5	55 46.5 12.5	38.5 ⑤ 46.5 76	6.5 ③ 7 11.5	11.5	88.5 ②	0	delay ④	3.1	Zn 76 Cu 18 Cr 20 Cd 1.0 Ni 30 Hg 0.05	32 Poly	1.0225
1–12 ④	Off Kawanoe Bottom (22.5)	85 ① 89.5 91	57 43.5 38	43 ④ 56.5 62	0 0 0	2	98 ①	0		3.1	Zn 147 Pb 55 Cu 40 Cr 35 Cd 2.5 Ni 33 Hg 1.67	146 Mol.	1.0193
24–2 ⑤	Off Mihara Bottom (5.0)	66.5 ④ 57.5 53	64.5 50.5 60	35 ⑤ 49.5 40	0.5 0 0	89	11 ⑤	0		2.0	Zn 235 Pb 115 Cu 30 Cr 23 Cd 3.0 Ni 19 Hg 0.39	4 Poly.	1.0194

Table 1. (continued)

Sample No.	Location (depth)	Fertiliz. membrane formation	First cleavage 1 cell	2 cell (normal)	multi-cell (polyspermy)	Gastrulation permanent blastula	gastrula (normal)	exo-gastrula	Other notes abnormal develop.	COD	heavy metals	benthos	specific density
	(m)	%	%	%	%	%	%	%		ppm	ppm		
24–5	Off Chigirijima	73.5	31	68	1	5.5	70.5	24	delay				
⑤	Isle	③ 63.5	42.5	④ 53.5	② 4		③	⑤	④	1.8			1.0170
	Surface	64.5	36	62.5	1.5								
		89.5	20	80	0	7.5	92.5	0				2	
②	Bottom (28.0)	① 96.5	7.5	① 92.5	0		②			1.1 (pH 9.2)		Poly.	1.0208
		93.5	12	86	2								
25–2	Off Kure Bottom	75	34.5	65.5	0	71.5	28.5	0			Zn 230 Pb 140 Cu 43 Cr 28 Cd 1.5 Ni 19 Hg 0.14	181 Poly.	1.0223
⑤	(13.0)	② 99	7	③ 93	0		⑤						
25–8	Off Otake	68.5	49	50	1	7	93	0					1.0195
⑤	Surface	③ 70.5	53	⑤ 46.5	0.5		②			17.1			
		84.5	82.5	17.5	0	7	93	0			Zn 1150 Pb 80 Cu 34 Cr 13 Cd 5.0 Ni 21 Hg 0.66	0	1.0240
⑤	Bottom (5.5)	② 76	76.5	⑤ 23.5	0		②						
		78.5	74	25.5	0.5								
30–5	Off Matsuyama	56.5	55.5	44.5	0	27.5	72.5	0			Zn 111 Pb 55 Cu 105 Cr 55 Cd 15.3 Ni 22 Hg 0.43	27 Poly.	1.0193
⑤	Bottom	⑤ 68.5	54	⑤ 45.5	0.5		③			2.3			
	(5.0)	74	49.5	49.5	1								

26–9 ⑤	Mitaziri Bay Surface	4.5 ⑤ 6.5 49	100 100 100	0 ⑤ 0 0	0 0 0					248 (pH 6.0)			1.0111
⑤	Bottom (4.0)	97 ① 98.5 88.5	64 67.5 62.5	36 ⑤ 32.5 37.5	0 0 0	6.5	93.5 ②	0		56	Zn 4900 Pb 215 Cu 130 Cr 25 Cd 3.0 Ni 21 Hg 0.77	0	1.0200
27–1 ⑤	Off Ube Surface	53.5 ④ 59.5 63.5	51.5 45 38	41.5 ⑤ 46.5 56.5	7 ③ 8.5 5.5	1	99 ①	0	delay ④	1.8			1.0196
④	Bottom (14.0)	98.5 ① 99 99.5	29 17.5 23.5	71 ② 82 76.5	0 36 0	36	64 ④	0		1.1	Zn 126 Pb 55 Cu 16 Cr 13 Cd 2.5 Ni 25 Hg 0.64	0	1.0208
29–5 ⑤	Off Tsurusaki Bottom (7.0)	96 ① 98 94.5	4.5 6 8	86.5 ② 78 82.5	9 ⑤ 16 9.5	4.5	94 ①	1.5	deform ④	1.3	Zn 109 Cu 34 Cr 13 Cd 2.3 Ni 25 Hg 0.34	79 Poly.	1.0233
29–6 ③	Off Saganoseki Bottom (3.0)	94 ① 96 94.5	8 5 8	91.5 ① 94 90.5	0.5 1 1.5	28.5	71.5 ③	0		1.9	Zn 503 Pb 1175 Cu 261 Cr 2503 Cd 7.8 Ni 1988 Hg 0.68	7 Crus.	1.0233
3–4 ①	Off Komatsujima Bottom (10.0) rough water	98 ① 78.5 98.5	4.5 30 3.5	94.5 ① 69 95	1 1 1.5	0.5	99.5 ①	0		1.4	Zn 140 Pb 50 Cu 55 Cr 45 Cd 1.8 Ni 57 Hg 0.17	20 Poly.	1.0215

Table 1. (continued)

Sample No.	Location (depth)	Fertiliz. membrane formation	First cleavage 1 cell	2 cell (normal)	multi-cell (polyspermy)	Gastrulation permanent blastula	gastrula (normal)	exo-gastrula	Other notes abnormal develop.	COD	heavy metals	benthos	specific density
	(m)	%	%	%	%	%	%	%		ppm	ppm		
C- 9	Running sea water of laboratory	96.5	8	92	0	0.5	99.5	0					
①		① 93.5	14	①86	0		①						1.0243
	Surface	97	5.5	94.5	0								
C-10	Running sea water of laboratory	95.5	9.5	90.5	0	1	99	0					
①		① 99	3	①97	0		①						1.0238
	Surface	98	4.5	95.5	0								

Table 2. Tentative ranking of inhibitory degrees of the polluted sea water upon the fertilization and further development of sea urchin eggs.

Inhibitory degree	Stage / Grade	Fertiliz. membrane formation %	First cleavage 1 cell %	2 cell (normal) %	multi-cell (polyspermy) * %	Gastrulation permanent blastula %	gastrula (normal) %	exo-gastrula * %	Remarks abnormal development **
Violently inhibitory sea water	5	0– 50	100– 50	0– 50	15–100	100– 50	0– 50	15–100	development stopped in early stages
Strongly inhibitory sea water	4	51– 59	49– 41	51– 59	11– 14	49– 36	51– 64	11– 14	development delayed or deformed
Moderately inhibitory sea water	3	60– 69	40– 31	60– 69	6– 10	35– 21	65– 79	6– 10	development somewhat delayed and deformed
Weakly inhibitory sea water	2	70– 84	30– 21	70– 79	2– 5	20– 6	80– 94	2– 5	—
Non-inhibitory ordinary sea water	1	85–100	20– 0	80–100	0– 1	5– 0	95–100	0– 1	—

*: Occurrences rather infrequent.
**: Noted when the abnormality or delay occurred on over 50% of the checked embryos.

Station numbers (=Sample No.) and location of respective stations

20—1 Osaka Bay
20—2 Off Kobe Harbour
20—3 Vicinity of Suma
20—4 Vicinity of Hayashizaki (Off Akashi Harbour)
20—5 Off the estuary of the River Kako
20—6 Off Nos. 1 and 2 Himeji Power Stations (Estuary of the River Ichi)
20—7 Entrance to Hirohata Bay
20—8 Off the factory of Nippon Steel Corporation
20—9 South-west off the factory of Nippon Steel Corporation
20-10 Estuary of the River Ibo. (Off the factory of Daicel Ltd.)
21—1 Middle of the distance between Iyeshima Island and the estuary of the River Ibo
21—2 West estuary of the River Ibo
21—3 Off the west estuary of the River Ibo
21—4 Cove of Iwami
21—5 Cove of Aioi
21—6 200 m off the factory of Sumitomo Cement Co. Ltd., Ako
21—7 Off the destination of a projected atomic power station, Kakuijima Island
21—8 Off a sulfuric acid factory, Inushima Isle
21—9 Off the factory of Teikoku Kako Co. Ltd., Kojima Bay (Entrance to Kojima Bay)
21—10 20 m off the factory of Mitsui Mining and Smelting Co. Ltd., Hibi
22—1 Tanoura Bay
22—2,3 Mizushima Bay
22—4 Mizushima Bay (Entrance to the Sound of Mizushima)
22—5,6 Mizushima Bay
22—7 Mizushima Bay, off Torishima Isle
22—8 to 10 Mizushima Bay
22—11 Mizushima Bay, off the factory of Kawasaki Steel Corporation
22—12, 13 Mizushima Bay
22—14 Mizushima Bay, off Mizushima
22—15, 16 Mizushima Bay
22—17 Mizushima Bay, 500 m off the factory of Kawasaki Steel Corporation
22—18 to 21 Mizushima Bay
22—22 Mizushima Bay, off Konko
22—23 to 26 Mizushima Bay
23—1 Mizushima Bay
23—2 Mizushima Bay, estuary of the River Takahashi
23—3 Mizushima Bay, south off the factory of Kawasaki Steel Corp.
23—4, 5 50 m off the factory of Nippon Kokan K.K., Fukuyama
23—6 Nippon Kokan Harbour, Fukuyama
23—7 Estuary of the River Ashida
23—8 Off the combined iron and steel works
24—1 30 m off the factory of Mitsubishi Heavy Industries, Ltd., Itozaki
24—2 Off the factory of Teijin Limited, Mihara
24—3 Off Tadanoumi
24—4 5 m off the factory of Mitsubishi Metal Mining Co. Ltd., Takehara
24—5 North-west off the factory of Toho Zinc Co. Ltd., Chigirijima Isle
24—6 Off the factory of Sankyo Kasei Co. Ltd.
24—7 Off combined factories, Nikata
24—8 Off east Nikata
24—9 Off west Nikata

24—10 Off the factory of Toyo Pulp Co. Ltd., Hiro
24—11 20 m off the factory of Toyo Pulp Co. Ltd., Hiro
24—12 150 m off the factory of Toyo Pulp Co. Ltd., Hiro
25—1 Middle of the distance between the factories of Nisshin Steel Co. Ltd. and Kobe Steel Ltd., Kure
25—2 Off Kure
25—3 Off an excreta plant, Kure
25—4 Waterway near the factory of Sailor Pen Co. Ltd.
25—5 Front of the factory of Ube Industries Ltd., Kaida Harbour
25—6 Off the shipyard of Mitsubishi Heavy Industries Ltd., Hiroshima
25—7 70 m off the factory of Mitsubishi Rayon Co. Ltd., Otake
25—8 50 m off the factories of Otake Paper Mills Co. Ltd. and Daicel Ltd., Otake
25—9 Off the factory of Koa Oil Co. Ltd.
25—10 50 m off the factory of Sanyo Pulp Co. Ltd., Iwakuni
25—11 100 m off the factories of Chugoku Electric Power Co. Inc. and Toyobo Co. Ltd., Iwakuni
25—12 Yanai Harbour
26—1 Off the factory of Nippon Steel Corporation, Hikari
26—2 Off the factories of Toyo Kohan Co. Ltd., Nippon Oil Co. Ltd. and Hitachi Shipbuilding & Engineering Co. Ltd., Kudamatsu
26—3 100 m off the factory of Idemitsu Oil Co. Ltd., Tokuyama
26—4 100 m off the factory of Japanese Geon Co. Ltd., Tokuyama
26—5 100 m off the factory of Tokuyama Soda Co. Ltd.
26—6 50 m off the factory of Toyo Soda Co. Ltd.
26—7 Off the factory of Sekisui Chemical Co. Ltd., Tokuyama
26—8 Off the factory of Sekaicho Rubber Co. Ltd., Tokuyama
26—9 Off the factories of Kyowa Hakko Kogyo Co. Ltd. and Kanebo Co. Ltd., Mitaziri Bay
27—1 200 m off the factory of Ube Industries Ltd., Ube
27—2 Off the chemical factory of Ube Industries Ltd.
27—3 300 m off the Power Station at the estuary of the River Koto
27—4 100 m off the factory of Onoda Cement Co. Ltd.
27—5 Off Onoda
27—6 Tajiri Harbour
28—1 South off Himejima Island
29—1 Estuary of the River Sumiyoshi, Ooita
29—2 West coast of Ooita Airport
29—3 Near the factory of Showa Denko K.K., Ooita
29—4 Off the Kyushu Oil Co. Ltd.
29—5 Off the Power Station, Tsurusaki
29—6 20 m off the factory of Nippon Mining Co. Ltd., Saganoseki
30—1 Off Iyo-Nagahama Harbour
30—2, 3 Off Iyo-Nagahama Harbour
30—4 200 m off the factory of Toray Industries Inc., Matsuyama
30—5 Off the factory of Maruzen Oil Co. Ltd., Matsuyama
31—1 Off the factory of Taiyo Oil Co. Ltd., Kikuma
31—2 Off the combined factories, the Kurushima Channel
31—3 30 m off the spinning factory, Imabari
31—4 20 m off the refinary works of Sumitomo Metal Mining Co. Ltd., Shisakajima Island
31—5 Off the Power Station and factory of Kuraray Co. Ltd., Saijo
31—6 Off the factory of Fuji Spining Co. Ltd., Niugawa
31—7 200 m off the refinary works of Sumitomo Light Metal Industries Ltd., Niihama
31—8 Off the factory of Sumitomo Chemical Co. Ltd., Niihama

31—9 Off the refinary works of Sumitomo Light Metal Industries Ltd., Kikumoto, Niihama.
1—1 Off Doi
1—2 Off Iyo-Mishima
1—3 Off the factory of Taio Paper M. Co. Ltd., Iyo-Mishima
1—4 Off Kawanoe
1—5 Off Kawanoe
1—6 Off the factory of Fuji Spining Co. Ltd., Toyohama
1—7, 8 Off Kannonji
1—9 3 km east from Ibukijima Island
1—10 Off Ibukijima
1—11 2 km south from Ibukijima Island
1—12 to 14 Off Kawanoe
1—15 Off Iyo-Mishima
1—16 200 m off the west pier of Iyo-Mishima Harbour
1—17 Off the estuary of the River Kinsei
1—18 Off Kawanoe
2—1 Off Misaki Point
2—2 Off the reclaimed land, Tadotsu.
2—3 Off the factory of Kawasaki Heavy Industry Co. Ltd., Sakaide Harbour
3—1 Southern part of the Sea of Harima
3—2 Estuary of the River Imagire
3—3, 4 Off Komatsujima
3—5 Off the Power Station, Anan